

Follow-up of the prevalence of hepatitis C virus genotypes in Spain during a nine-year period (1996-2004)

José Manuel Echevarría, Pilar León, Francisco Pozo and Ana Avellón

Servicio de Microbiología Diagnóstica. Centro Nacional de Microbiología. Instituto de Salud Carlos III. Majadahonda. Madrid. Spain.

BACKGROUND. Recent data suggest that the prevalence of genotype 4 HCV strains among Spanish carriers is increasing.

OBJECTIVE. To assess changes in the prevalence of HCV genotypes in Spain during the last nine years.

METHODS. HCV RNA was amplified by the polymerase chain reaction from 3161 serum samples from unselected, anti-HCV-positive individuals, and the HCV genotype was identified by a reverse hybridisation assay (line probe assay, LiPA). Samples came from 17 different regions of Spain and were obtained between January, 1996 and December, 2004.

RESULTS. The overall prevalence of HCV genotypes was: 1b, 41.3%; 1a, 24.1%; 3, 19.6%; 4, 11.6%; 2, 3.1%; and 5, 0.3%. The prevalence of genotypes 1a, 3 and 4 increased significantly among patients born after 1950, and that of genotype 1b decreased among them. These significant differences in regard to age were also observed among patients lacking notified high-risk factors. A main switch-up in prevalence of genotypes 1a and 3 was found when patients born in 1941-1950 were compared with those born in 1951-1960, but the same finding in genotype 4 was delayed to patients born in 1961-1970.

CONCLUSIONS. Two separate epidemics of HCV seem to have occurred in Spain during the last 30 years. The former one involved the spread of HCV genotypes 1a and 3. The second was more recent, and involved the spread of genotype 4.

Key words: Hepatitis C virus. Hepatitis C virus genotypes. Hepatitis C virus genotype 4. Molecular epidemiology.

Seguimiento de la prevalencia de genotipos del virus de la hepatitis C en España durante nueve años (1996-2004)

ANTECEDENTES. Algunos datos recientes sugieren que la prevalencia del genotipo 4 del virus de la hepatitis C (VHC) se ha incrementado en España en los últimos años.

OBJETIVO. Poner de manifiesto cambios en la prevalencia de los genotipos del VHC en España durante los últimos 9 años.

MÉTODOS. Se amplificó un fragmento del genoma del VHC presente en 3.161 muestras de suero tomadas de individuos positivos para anti-VHC no seleccionados. Después se identificó el genotipo mediante una prueba de hibridación reversa (line probe assay, LiPA).

Las muestras procedían de 17 regiones diferentes y se tomaron entre enero de 1996 y diciembre de 2004.

RESULTADOS. La prevalencia global de genotipos del VHC fue: 1b, 41,3%; 1a, 24,1%; 3, 19,6%; 4, 11,6%; 2, 3,1%; y 5, 0,3%. Considerando la edad de los pacientes, las prevalencias de los genotipos 1a, 3 y 4 fueron significativamente mayores entre los nacidos después de 1950, en tanto que la del genotipo 1b fue menor.

Estas diferencias significativas se observaron también al considerar sólo a los pacientes sin factores de alto riesgo conocidos. Los incrementos significativos en las prevalencias de los genotipos 1a y 3 se encontraron al comparar entre sí a los pacientes nacidos en 1941-1950 con los nacidos en 1951-1960. Para el genotipo 4, este hallazgo se produjo, sin embargo, al comparar estos últimos con los nacidos en 1961-1970.

CONCLUSIONES. En España parecen haber sucedido dos episodios recientes de importación de cepas del VHC. El primero supuso la diseminación de cepas de los genotipos 1a y 3. El segundo fue más reciente y supuso la introducción de cepas del genotipo 4.

Palabras clave: Virus de la hepatitis C. Genotipos del VHC. Genotipo 4 del virus de la hepatitis C. Epidemiología molecular.

Correspondencia: Dr. J.M. Echevarría.
Servicio de Microbiología Diagnóstica.
Centro Nacional de Microbiología.
Instituto de Salud Carlos III.
Ctra. Majadahonda-Pozuelo, km 2.
28220 Majadahonda. Madrid. Spain.
Correo electrónico: jmecheva@isciii.es

Manuscrito recibido el 27-10-2004; aceptado el 29-4-2005.

Introduction

Hepatitis C virus (HCV) strains are grouped into six main genotypes, namely genotypes 1 to 6, and into several subtypes within most of them¹. Genotype 1a prevails

TABLE 1. Geographical and temporal distribution of the samples included in the study

Region	Number of centres	Number of samples in year									Total
		1996	1997	1998	1999	2000	2001	2002	2003	2004	
Andalucía	10	2	4	14	13	31	178	165	81	88	576
Aragón	2	6	1	12	—	—	—	—	—	—	19
Asturias	1	—	—	—	1	—	1	—	—	—	2
Baleares	3	—	—	4	4	20	102	108	58	29	325
Canarias	1	—	—	—	—	2	—	—	—	—	2
Cantabria	3	—	—	—	—	—	13	47	12	1	73
Castilla-La Mancha	3	22	9	18	25	37	72	83	67	41	374
Castilla y León	3	3	2	13	83	87	29	35	76	15	343
Cataluña	4	16	3	4	28	16	8	5	5	11	96
Ceuta	1	—	1	—	12	41	32	36	31	24	177
Extremadura	5	—	—	2	8	68	35	27	66	74	280
Galicia	3	1	—	3	7	30	9	22	29	16	117
Madrid	11	3	1	7	129	218	122	59	7	5	551
Murcia	1	—	—	—	—	—	1	2	—	—	3
Navarra	1	—	—	2	1	—	1	—	—	—	4
País Vasco	1	—	—	—	1	—	—	—	1	—	2
Valencia	5	—	4	57	5	1	—	2	1	1	71
Total	58	53	25	136	317	551	603	591	434	305	3.015

TABLE 2. Distribution of HCV genotypes among patients with notified HIV infection or history of IVDU (group 1), children born from carrier mothers (group 2), immigrants (group 3), and native Spanish patients lacking notified risk factors (group 4)

Group	Number of samples displaying HCV genotype (%)						Total
	1a	1b	2	3	4	5	
1	173 ^a (34.0)	89 (17.5)	8 (1.6)	140 ^b (27.5)	98 ^c (19.3)	1 (0.2)	509
2	11 (37.9)	9 (31.0)	—	4 (13.8)	5 (17.2)	—	29
3	25 (25.5)	29 (29.6)	11 (11.2)	21 (21.4)	12 (12.2)	—	98
4	519 ^a (21.8)	1119 (47.0)	73 (3.1)	425 ^b (17.9)	235 ^c (9.9)	8 (0.3)	2379
Total	728 (24.1)	1246 (41.3)	92 (3.1)	590 (19.6)	350 (11.6)	9 (0.3)	3015

^achi² = 33.43, p < 0.01.^bchi² = 24.15, p < 0.01.^cchi² = 35.21, p < 0.01.

in America and in the North and centre of Europe, whereas genotype 1b prevails in Eastern Europe, the Far East and the Mediterranean. Genotype 3 strains originating from the Indian subcontinent have spread broadly among injection drug users of the Western hemisphere². Genotype 4 is thought original from Africa and has shown a significant prevalence in some European countries from the Mediterranean basin³. Two main episodes of spread of HCV are thought to have occurred in Europe: the former one involved the spread of genotype 1 strains through blood transfusion, and the second followed the introduction of genotype 3 strains through the use of injection drugs².

Genotype 1b has been found prevalent in all studies performed on unselected chronic carriers from Spain, always

TABLE 3. Distribution of HCV genotypes among immigrants in regard to the region of origin

Region	Number of samples displaying HCV genotype (%)					Total
	1a	1b	2	3	4	
Morocco	17 (30.4)	16 (28.6)	5 (8.9)	10 (17.9)	8 (14.2)	56
Tropical Africa	8 (29.6)	6 (22.2)	6 (22.2)	4 (14.8)	3 (11.1)	27
Eastern Europe	—	6	—	4	1	11
Latin América	—	1	—	—	—	1
India	—	—	—	3	—	3
Total	25 (25.5)	29 (29.6)	11 (11.2)	21 (21.4)	12 (12.2)	98

reaching figures above 50%⁴⁻⁷. Genotype 3 prevalence ranged from 4 to 17%, rising above 20% among injection drug users⁴⁻⁸. Prevalence of genotype 4 has been usually found below 10%, but higher figures have been recorded among injection drug users⁹⁻¹¹. These data suggest that the import of genotype 3 strains into Spain would have been accompanied by the introduction and spread of genotype 4. The aim of the present work was to assess changes in the prevalence of HCV genotypes in Spain along the period 1996-2004.

Materials and methods

The study was retrospective in nature and included 3161 individual, HCV RNA-positive serum samples from HCV carriers sent to a reference laboratory for genotype identification prior to antiviral treatment. Samples from patients suspected to have been involved in epidemic outbreaks of HCV infection were not included, and no additional selection in regard to age, sex, geographical origin, other risk

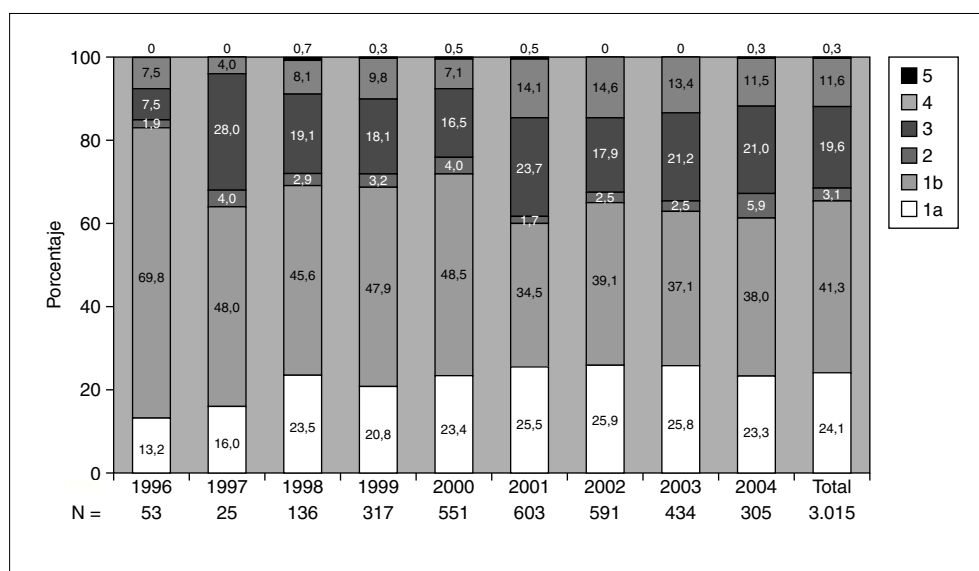


Figure 1. Prevalence of HCV genotypes among 3,015 samples from unselected carriers from Spain, grouped by time of sampling.

TABLE 4. Distribution of HCV genotypes found on samples studied during two periods of time

HCV genotype	Number of samples in years (%)		Total (%)
	1996-2000	2001-2004	
1a	238 (22.0)	490 (25.3)	728 (24.1)
1b ^a	530 (49.0)	716 (37.0)	1,246 (41.3)
2	38 (3.5)	54 (2.8)	92 (3.1)
3	185 (17.1)	405 (21.0)	590 (19.6)
4 ^b	86 (7.9)	264 (13.7)	350 (11.6)
5	5 (0.5)	4 (0.2)	9 (0.3)
Total	1,082	1,933	3,015

^achi² = 40.31, p < 0.01.

^bchi² = 21.48, p < 0.01.

factors for acquisition of HCV infection or any other parameter was done. After performing genotyping tests, 3015 samples were considered for analysis (see Results).

Samples came from 58 health care institutions (21 reference hospitals, 28 regional hospitals, four transfusion centres, four private institutions and one reference laboratory) from 17 different regions of Spain (table 1) and were taken between January, 1996 and December, 2004. Most samples (2743, 90.9%) were from eight regions contributing with more than 100 samples each. Infection by human immunodeficiency virus (HIV) and/or history of intravenous drug use (IVDU) was notified in 509 cases (group 1). Among the remaining, 29 samples were from children born from HCV-infected mothers (group 2); 98 were from immigrants (group 3); and 2379 were from adults native from Spain (group 4). The gender was known from all patients but eight, with a predominance of men (67.8%). Age was known from 2014 patients (66.8%), and ranged from newborn age to 89 years old.

A fragment of the 5' non-coding region of the HCV genome was amplified by the polymerase chain reaction (PCR)⁶ from all samples, and the genotype was determined by reverse hybridisation (line probe assay, LiPA), using a commercial test (Versant HCV GenoAmp LiPA, Bayer Healthcare LLC, Tarrytown, NY, USA). Prevalence of genotypes 1a, 1b, 2, 3, 4, 5 and 6 was calculated, and results from samples grouped by different criteria were compared by the chi square (chi²) test. Differences were considered statistically significant for p < 0.01.

Results

HCV genotype 1 strains present in 146 samples displayed hybridisation patterns that did not allow a genosubtype assignment, and were excluded from the analysis. These samples accounted for 4.6% of the total and were almost uniformly distributed along the period of the study (range: 4.1 to 4.8% per year). Table 2 summarises the results obtained on the remaining 3015 samples, according to the four groups of patients studied. Table 3 details those corresponding to immigrants in regard to the region of origin. Genotypes 1a, 3 and 4 were significantly more prevalent among patients from group 1 in comparison with those from group 4. Prevalence of genotype 2 was significantly higher among immigrants from the tropics of Africa in comparison with the remaining patients (22.2% vs 2.9%; chi² = 27.62, p < 0.01).

Figure 1 shows the overall prevalence of HCV genotypes on patients grouped by the year of sampling. Genotype 1b was always the more prevalent, but its overall prevalence was just 41.3%. Since a increasing trend was observed for the overall prevalence of genotypes 1a and 4, a comparison was done with samples grouped into two periods of time (1996-2000 and 2001-2004) (table 4). A significant increase of the prevalence of genotype 4 was found, together with a decrease of the prevalence of genotype 1b.

Table 5 shows the prevalence of HCV genotypes in regard to the year of birth of the patients. Genotype 1b was significantly more prevalent among those born before 1951, and the prevalence of genotypes 1a, 3 and 4 was significantly higher among patients born after 1950 (table 6). Similar findings in regard to age were found among adult patients lacking notified HIV infection and history of IVDU (tables 7 and 8). Among the latter, a significant switch-up of the prevalence of genotypes 1a and 3 was observed among patients born in 1951-1960 in comparison with those born before 1951 (7.7% vs. 19.3%, chi² = 20.39, p < 0.01 for genotype 1a; 2.6% vs. 15.6%, chi² = 39.17, p < 0.01 for genotype 3). For genotype 4, such switch-up in prevalence was, however, delayed to patients born in 1961-1970 (4.6% vs. 14.5%, chi² = 37.99, p < 0.01).

TABLE 5. Distribution of HCV genotypes in regard to the time of birth of the patients

HCV genotype	Patients born between (%)								Total (n,%)
	1910-1930	1931-1940	1941-1950	1951-1960	1961-1970	1971-1980	1981-1990	1991-2004	
1a	8 (8.2)	12 (7.7)	19 (8.9)	76 (22.2)	266 (31.2)	93 (30.1)	4 (17.4)	8 (34.8)	486 (24.1)
1b	78 (80.4)	126 (81.3)	171 (80.3)	149 (43.7)	202 (23.7)	91 (29.4)	14 (60.9)	7 (30.4)	838 (41.6)
2	5 (5.2)	9 (5.8)	9 (4.2)	18 (5.3)	21 (2.5)	3 (1.0)	1 (4.3)	—	66 (3.3)
3	1 (1.0)	2 (1.3)	9 (4.2)	64 (18.8)	226 (26.5)	67 (21.7)	3 (13.0)	4 (17.4)	376 (18.7)
4	5 (5.2)	5 (3.2)	5 (2.3)	31 (9.1)	136 (15.9)	55 (17.8)	1 (4.3)	4 (17.4)	242 (12.0)
5	—	1 (0.6)	—	3 (0.9)	2 (0.2)	—	—	—	6 (0.3)
Total	97	155	213	341	853	309	23	23	2,014

TABLE 6. Distribution of HCV genotypes among patients born before or after 1950

HCV genotype	Patients born between (%)		Total (%)
	1910-1950	1951-2004	
1a ^a	39 (8.4)	447 (28.9)	486 (24.1)
1b ^b	375 (80.6)	463 (29.9)	838 (41.6)
2 ^c	23 (4.9)	43 (2.8)	66 (3.3)
3 ^d	12 (2.6)	364 (23.5)	376 (18.7)
4 ^e	16 (3.4)	227 (14.7)	242 (12.0)
5	1 (0.2)	5 (0.3)	6 (0.3)
Total	465	1,549	2,014

^achi² = 80.74, p < 0.01.^bchi² = 377.11, p < 0.01.^cchi² = 4.65, p < 0.05.^dchi² = 101.69, p < 0.01.^echi² = 41.00, p < 0.01.

TABLE 7. Distribution of HCV genotypes among patients lacking notified HIV infection, nor history of IVDU, born before or after 1950

HCV genotype	Patients born between (%)		Total (%)
	1910-1950	1951-2004	
1a ^a	35 (7.7)	295 (27.0)	330 (13.9)
1b ^b	370 (81.3)	371 (34.0)	741 (31.3)
2 ^c	23 (5.1)	31 (2.8)	54 (2.3)
3 ^d	12 (2.6)	241 (22.1)	253 (10.7)
4 ^e	14 (3.1)	148 (13.6)	162 (6.8)
5	1 (0.2)	5 (0.5)	6 (0.3)
Total	455	1,091	1,546

^achi² = 70.44, p < 0.01.^bchi² = 286.11, p < 0.01.^cchi² = 4.03, p < 0.05.^dchi² = 87.36, p < 0.01.^echi² = 36.55, p < 0.01.

Discussion

Data regarding prevalence of HCV genotypes in Europe suggest that genotype 3 produced a recent epidemic of HCV infection among the injection drug users and spread subsequently to other population groups that had been already penetrated by genotype 1 strains, mainly by genotype 1b, in the past^{2,12-14}.

The present investigation is affected by limitations that are characteristic of retrospective studies, such as the presence of uncontrolled sampling variations from year to year, the lack of representativity of the sample in terms of population, or the risk of underreporting of relevant epidemiological data for the patients included. With these limitations in mind, the results show that Spanish HCV carriers born before 1951 are mainly infected by genotype 1b. In addition, they show that genotype 1a and 3 strains are significantly more frequent among patients born after 1950, and confirm the high prevalence of these genotypes among patients displaying significant risk factors, such as HIV co-infection or history of IVDU. Since the acquisition of the HCV infection is very uncommon before the age of 20, this is consistent with the introduction, 20-30 years ago, of genotype 1a and 3 strains into the IVDU population and with its spread to other population groups. Evidence for such introduction of HCV genotype 1a together with

genotype 3 has been also reported from other European countries¹²⁻¹⁴.

In agreement with prior findings⁹⁻¹¹, the age-specific patterns of prevalence found for genotype 4 indicate that this HCV genotype has been also introduced recently in Spain. They suggest, in addition, that the import of this genotype was independent of the import of genotypes 1a and 3 and happened around 10 years later. In support of this interpretation, an increasing prevalence of genotype 4 among the youngest HCV carriers have been reported from Italy¹⁵⁻¹⁷, and a significant association of this genotype with infections acquired after 1990 has been found in Austria¹⁸.

It seems very likely that introduction of genotype 4 into Europe and the United States has been related with injection drug use^{15,19,20}, and the findings of this investigation agree with this proposal. The significant increase in prevalence of genotype 4 found among patients without notified high risk factors born after 1960 suggest, in addition, that this genotype begun to spread to other population groups at the beginning of the 80's decade.

The diversification observed for genotypes 1 and 2 in Africa²¹ and the geographical distribution found for genotypes 4 and 5 lead to suggest an African origin for all these four HCV genotypes. Import of African strains of hepatitis B virus (HBV) into Spain has been recently do-

TABLE 8. Distribution of HCV genotypes in regard to the time of birth among patients with (group 1) or without (group 4) notified HIV infection or history of IVDU

Patients born between	Group	HCV genotype (%)						Total
		1a	1b	2	3	4	5	
1910-1930	1	—	—	—	—	—	—	0
	4	8 (8.2)	78 (80.4)	5 (5.2)	1 (1.0)	5 (5.2)	—	97
1931-1940	1	1	—	—	—	—	—	1
	4	11 (7.1)	126 (81.8)	9 (5.8)	2 (1.3)	5 (3.2)	1 (0.6)	154
1941-1950	1	—	3	—	—	—	—	3
	4	16 (7.8)	166 (81.4)	9 (4.4)	9 (4.4)	4 (2.0)	—	204
1951-1960	1	23 (36.5)	7 (11.1)	1 (1.6)	21 (33.3)	11 (17.5)	—	63
	4	52 (19.3)	139 (51.5)	15 (5.6)	42 (15.6)	19 (7.0)	3 (1.1)	270
1961-1970	1	84 (33.6)	43 (17.2)	5 (2.0)	68 (27.2)	50 (20.0)	—	250
	4	178 (30.3)	155 (26.4)	13 (2.2)	153 (26.1)	85 (14.5)	2 (0.3)	586
1971-1980	1	28 (35.4)	20 (25.3)	—	20 (25.3)	11 (13.9)	—	79
	4	65 (29.0)	68 (30.4)	3 (1.3)	44 (19.6)	44 (19.6)	—	224
1981-1990	1	1	2	—	—	—	—	3
	4	—	9	—	2	—	—	11
Total	1	137 (34.3)	75 (18.8)	6 (1.5)	109 (27.3)	72 (18.0)	—	399
	4	330 (21.3)	741 (47.9)	54 (3.5)	253 (16.4)	162 (10.5)	6 (0.4)	1,546

cumented by the finding of genotype A/ayw1 and genotype E strains among native Spanish carriers^{22,23}. It seems, therefore, likely that introduction of African HBV and HCV strains into the Spanish population have been epidemiologically related. It is thought that the import of HCV genotype 3 and HBV genotype D/ayw3 strains original from India into the European population of IVDU were also two epidemiologically related events.

Acknowledgments

The authors wish to thank Teresa Arias, Consuelo Elola, José A. López and Inés Parera for their excellent technical assistance, as well as the following health care institutions, which sent the samples used in the study: Hospital de la Inmaculada, Hospital Comarcal de Ronda, Hospital Comarcal de La Línea de la Concepción, Hospital Infanta Helena, Hospital San Juan de la Cruz, Hospital de San Agustín, Hospital de Poniente, Hospital Virgen de las Nieves, Hospital de la Axarquía and Centro Regional de Transfusión Sanguínea de Málaga, Andalucía; Hospital San Jorge and Hospital Miguel Servet, Aragón; Hospital de San Agustín, Asturias; Hospital Son Dureta, Hospital Virgen de Montetoro and Policlínica Miramar, Baleares; Hospital Virgen de Candelaria, Canarias; Hospital Marqués de Valdecilla, Hospital Comarcal Sierrallana and Banco de Sangre de Cantabria, Cantabria; Hospital General Universitario de Guadalajara, Hospital de Santa Bárbara and Hospital Comarcal de Hellín, Castilla-La Mancha; Hospital del Bierzo, Hospital General de Segovia and Hospital General de Soria, Castilla y León; Hospital Comarcal de Palamós, Hospital Comarcal de Figueres, Laboratori de Referència de Catalunya and Clínica Renal Rotellar, Cataluña; Centro de Donación de Sangre de la Cruz Roja, Ceuta; Hospital Ciudad de Coria, Hospital Virgen del Puerto, Hospital Comarcal de Llerena, Hospital Campo Arañuelo and Complejo Hospitalario de Cáceres, Extremadura; Hospital da Costa, Complejo Hospitalario de Pontevedra and Hospital de Valdeorras, Galicia; Fundación Hospital de Alcorcón, Hospital de La Princesa, Hospital La Paz, Hospital de Getafe, Hospital Gregorio Marañón, Hospital Niño Jesús, Hospital Gómez Ulla, Fundación Jiménez Díaz, SaniLab, Banco de Sangre de la Cruz Roja and Centro de Transfu-

sión de la Comunidad de Madrid; Hospital Virgen de la Arrixaca, Murcia; Hospital de Navarra; Banco de Sangre de Guipúzcoa, País Vasco; Hospital General de Elche, Hospital Comarcal de San Juan, Hospital Comarcal de Villajoyosa, Hospital General de Alicante and Hospital General de Castellón, Valencia.

References

1. Simmonds P, Holmes EC, Cha TA, Chan SW, McOmish F, Irvine B, et al. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS5 region. *J Gen Virol.* 1993;74: 2391-9.
2. Pawlotsky JM, Tsakiris L, Roudot-Thoraval F, Pellet C, Stuyver L, Duval J, et al. Relationship between hepatitis C virus genotypes and sources of infection in patients with chronic hepatitis C. *J Infect Dis.* 1995;171: 1607-10.
3. Ravaggi A, Rossini A, Mazza C, Puoti M, Marin MG, Cariani E. Hepatitis C virus genotypes in Northern Italy: clinical and virological features. *J Clin Microbiol.* 1996;34:2822-5.
4. Domingo MJ, Fernández I, Lumberras C, Manzaneres J, Morales JM, Noriega AR, et al. Utilización de la hibridación de secuencias de DNA con sondas específicas de oligonucleótidos para identificar genotipos del virus de la hepatitis C. *Enferm Infecc Microbiol Clin.* 1996;14:433-6.
5. Pena MJ, Mosquera MM, Pérez MC, Rodríguez San Román JL, Martín JM, Avalos O, et al. Prevalencia de genotipos del virus de la hepatitis C: epidemiología y características histológicas. *Enferm Infecc Microbiol Clin.* 1998; 16:456-60.
6. León P, López JA, Amela C, Elola C, Echevarría JM y el Grupo Español de Estudio de Donantes de Sangre en Riesgo de Transmisión del VHC (GEDSRT-VHC). Prevalencia de tipos del virus de la hepatitis C en donantes de sangre españoles: resultados de un estudio multicéntrico a nivel estatal. *Enferm Infecc Microbiol Clin.* 1999;17:448-53.
7. Arenas MD, Muñoz C, Sánchez-Payá J, Jover R, Egea JJ, Gil MT, et al. Distribución de genotipos del virus de la hepatitis C en una población de hemodiálisis en la provincia de Alicante. *Nefrología.* 2001;21:581-7.
8. Touceda S, Pereira M, Agulla A. Prevalencia de genotipos del virus de la hepatitis C en el área de El Ferrol (La Coruña, España). *Enferm Infecc Microbiol Clin.* 2002;20:200-4.
9. Sánchez-Quijano A, Abad MA, Torronteras R, Rey C, Pineda JA, Leal M, et al. Unexpected high prevalence of hepatitis C genotype 4 in Southern Spain. *J Hepatol.* 1997;27:25-9.

10. Ramos MC, Torío R, Mazón MA, Martín Gil FJ, Del Álamo M. Hepatitis C virus genotype 4 in a North-west Spain district. *J Clin Virol.* 2003;28:223-4.
11. Marcos F, Albo MI, Arbol F, Gómez-Soto FM, Blanco S, Viana A. Genotipo 4 del virus de la hepatitis C en una población de pacientes con infección por virus de la inmunodeficiencia humana. *Gastroenterol Hepatol.* 2004;27:388-9.
12. Ross RS, Viazov S, Renzing-Kohler K, Roggendorf M. Changes in the epidemiology of hepatitis C infection in Germany and in the predominance of hepatitis C subtypes. *J Med Virol.* 2000;60:122-5.
13. De Moreau de Gerbey AI, Bodeus M, Goubau P. Age trend in hepatitis C virus genotype distribution as seen in a Brussels teaching hospital. *Acta Clin Belg.* 2001;56:220-4.
14. Dal Molin G, Ansaldi F, Biagi C, D'Agaro P, Comar M, Croce L, et al. Changing molecular epidemiology of hepatitis C virus infection in Northeast Italy. *J Med Virol.* 2002;68:352-6.
15. Argentini C, Dettori S, Villano U, Guadagnino V, Infantolino D, Dentico P, et al. Molecular characterisation of HCV genotype 4 isolates circulating in Italy. *J Med Virol.* 2000;62:84-90.
16. Matera G, Lamberti A, Quirino A, Foca D, Giancotti A, Barreca GS, et al. Changes in the prevalence of hepatitis C virus (HCV) genotypes in Calabria, Southern Italy. *Diagn Microbiol Infect Dis.* 2002;42:169-73.
17. Cenci M, De Soccio G, Recchia O. Prevalence of hepatitis C virus (HCV) genotypes in central Italy. *Anticancer Res.* 2003;23:5129-32.
18. Haushofer AC, Koptý C, Hauer R, Brunner H, Halbmayer WM. HCV genotypes and age distribution in patients of Vienna and surrounding areas. *J Clin Virol.* 2001;20:41-7.
19. Van Asten L, Verhaest I, Lamzira S, Hernández-Aguado I, Zangerle R, Boufassa F, et al. Spread of hepatitis C virus among European injection drug users infected with HIV: a phylogenetic analysis. *J Infect Dis.* 2004;189:292-302.
20. Lyra AC, Ramrakhiani S, Bacon BR, Di Bisceglie AM. Infection with hepatitis C virus genotype 4 in the United States. *J Clin Gastroenterol.* 2004;38:68-71.
21. Jeannel D, Fretz C, Traore Y, Kohdjo N, Bigot A, Pe Gamy E, et al. Evidence for high genetic diversity and long-term endemicity of hepatitis C virus genotypes 1 and 2 in West Africa. *J Med Virol.* 1998;55:92-7.
22. Echevarría JM, León P. Hepatitis B virus genotypes identified by a Line Probe Assay (LiPA) among chronic carriers from Spain. *Enferm Infecc Microbiol Clin.* 2004;22:452-4.
23. Echevarría JM, Avellón A, Magnius LO. Molecular epidemiology of hepatitis B virus in Spain: identification of viral genotypes and prediction of antigenic subtypes by limited sequencing. *J Med Virol.* 2005;76:176-84.